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THE USE OF COLCHICINE IN PLANT BREEDING

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## THE USE OF COLCHICINE IN PLANT BREEDING

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Colchicine, a poisonous medicinal chemical, has been used since 1937 in plant breeding work to produce changes in plants by doubling the number of chromosomes in cells, a condition referred to as polyploidy. The increased number of chromosomes usually brings about an increase in size of the affected cells and various degrees of changes in their functions. In contrast with the normal plants, those developed by colchicine treatment often show changes in height and width; in thickness of branches; in size, shape, and texture of leaves, flowers, fruits, and seeds; in fertility of flowers; and in physiological responses. However, the degree of changes produced when the chromosome number is doubled cannot be predicted, and "magic" changes referred to by some popular writers are not to be expected. The visual changes induced in some plants are so small that even an experienced person will have difficulty in recognizing them.

Colchicine is extracted from either the seeds or the corms of Colchicum autumnale L. (meadow saffron or fall crocus) and may be bought in very small quantities in powder form from certain chemical concerns mentioned at the end of this article. A small quantity of colchicine can be used to treat a large number of plants, seeds, or other types of material, if applied without undue waste. Where a dipping method is used, the solution may be used several times. Generally colchicine is used in water solution in concentrations from as low as 0.1 percent up to 1.00 percent; it also can be used in 10-percent glycerine in water solution. The assistance of a druggist may be obtained in preparing desired percentages and quantities of solutions.

In handling colchicine, extreme care should be taken to--

- . Keep it out of the eyes to avoid possible dangerous consequence.
- . Wash hands after contact with the chemical to prevent possible skin irritation.

### HOW COLCHICINE WORKS

The colchicine solution affects plant cells during the division stages and has no apparent effect on nondividing cells. In normal cell divisions the chromosomes split lengthwise, and each half chromosome migrates to the opposite sides of the cell. A new cell wall forms between the two masses of chromosomes and thus two daughter cells are formed, each having the same number of chromosomes as the mother cell. If colchicine is

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absorbed into the dividing cell, the chromosomes split but the split halves do not migrate to the opposite sides, and the division of the cell is prevented. Thus, from the colchicine treatment there results a cell with twice as many chromosomes in its nucleus as the same cell had previous to treatment. After such initial chromosome doubling, if the colchicine diffuses away from the plant tissues or becomes so dilute as to be ineffective, the nuclei in the cells with the double chromosome number can divide normally to form daughter cells but with the double number of chromosomes. In addition to doubling the number of chromosomes, colchicine treatment has at times been found to cause addition or loss of one or more chromosomes in both the normal and the doubled types. Some abnormalities appearing on some treated plants may be caused by it. It is suspected that colchicine may also cause gene mutation, but there is considerable doubt about this. It is obvious from the way colchicine affects plant cells that applications will be effective if the material to be treated is growing vigorously, so that many cells are dividing. Treatment of dormant seeds, scales of lily bulbs, and similar material, however, has induced polyploidy, presumably because colchicine penetrates the seeds and scales and is maintained there for some time until cell divisions have begun; after this the colchicine becomes effective.

#### TYPES OF CHANGES INDUCED

In the terminal growing points of shoots and in lateral buds, there appear to be three cell layers each of which gives rise to a separate tissue or a tissue system or to areas in a tissue in the stem, leaves, flowers and fruits. The outer cell layer, 1st layer (L-I) of the growing point of the shoot or bud generally gives rise to a one-cell thick tissue, the epidermis. The next layer, 2nd layer (L-II) may give rise, depending on the kind of plant, to either a layer of tissue one-cell thick immediately under the epidermis, or to several layers of the cortical tissue. A third layer (L-III) may give rise to the remaining tissues in the stem. Pollen grains and egg cells originate in the tissue of anthers and ovules, respectively, derived from L-II. For a fuller discussion of this subject, the reader is referred to articles 5 and 8 in the list of the literature given at the end of this article.

Colchicine treatment may affect one cell layer and not the others, or any two layers may be affected and the remaining layer not be affected, or all three may be affected. This last possibility is rarer than the first two. Furthermore, many of these layers may have become only sectorially polyploid. Experiments have shown that polyploidy resulting from colchicine treatment may be of three main types:

1. Epidermal-polyploidy. Enlargement of stomates (breathing pores) on leaves subsequent to treatment indicates that the epidermis or outermost layer of the material is polyploidized, but it does not indicate that internal tissues are changed. If the internal tissues are not polyploidized, this type of change may be called epidermal-polyploidy.

2. Internal-polyploidy. Any change in which a layer of cells or a tissue or tissues beneath the epidermis are made polyploid but in which the epidermis remains normal may be called internal-polyploidy. This type of polyploidy may be of three kinds: (a) only the tissue immediately next to the epidermis is polyploidized; (b) the inner tissues are polyploidized, but the tissue for a number of cell layers beneath the epidermis remains normal; (c) all the inner tissues become polyploidized, the epidermis of the plant alone remaining normal. Enlarged pollen grains from flowers of a treated plant indicate that at least the tissue next to the epidermis is polyploidized from the colchicine treatment. Leaves become larger and broader in any kind of internal polyploidy. Flower petals and especially the anthers are chiefly affected if the tissue next to the epidermis is polyploidized. Chromosome counts in the cells of roots of a vegetative cutting would show whether or not the conductive tissue of the stem is polyploid. Such an examination of roots of seedlings treated by colchicine does not indicate whether the aboveground portion of a seedling is affected by colchicine treatment or not.

3. Total-polyploidy. A plant in which all the tissues are polyploidized may be designated as total-polyploid.

The extent of polyploidy can best be determined by examining, under a high-power microscope, paraffin sections of treated material taken from the very tip of a vigorously growing branch suspected of polyploidy. In such preparations, one must determine the chromosome number in a number of cells in each tissue system mentioned above to find out exactly what tissue system is polyploidized. Size of cells or of nuclei or of mere chromatin mass alone is not sufficient to determine the absolute extent of polyploidy in the treated plant.

From the results on induced polyploids it has been shown that the degree of fertility of these plants is usually affected. In a number of cases sterile hybrids resulting from hybridization between species of the same or closely related genera have been made fertile by chromosome doubling, while with others that has not been possible. Often where the plants have been highly fertile, they are made less fertile. Lessening of the fertility or inducing complete sterility by polyploidy may not always be deleterious. Where, for instance, plants are propagated vegetatively and grown for leaves and flowers or other plant parts, sterility may even be desirable. In such fruits as strawberries and raspberries, lowering of fertility causes numerous seeds to fail to develop and results in partly developed and deformed fruits. However, such plants may become of great value as breeding material.

A number of reports indicate that besides quantitative changes in plants resulting from polyploidy there also have been changes of qualitative character. These may be in color intensity of leaves and flowers; in fragrance of leaves, flowers, or other plant parts; and in content of oils, starches, sugars, and vitamins. Fundamentally these changes that are ordinarily considered qualitative are mostly, if not entirely, changes of quantitative nature, since they result from either an increase or a decrease of the various products naturally present in the plants.

#### METHODS OF TREATMENT AND DETECTION OF POLYPLOIDY

The colchicine solution at the beginning and during the time of treatment should be at room temperature. With small seedlings or fast-growing herbaceous plants it is best that the treatment be made with as weak a solution as may be effective, as early in the active growth of the plant as possible, and for a relatively short period of time. Application of a strong solution or a prolonged treatment may prevent growth of the treated material and kill it. Success in the treatment of growing material is based on the principle that the chemical should reach the regions of growth; therefore, the manner of treatment depends upon the type of material to be treated. A few of the methods, which may be modified to suit the material and the inclination of each experimenter, follow:

1. Seed treatment. Seeds of many varieties of plants have been soaked in a 0.2 percent to 1.6 percent (usually 0.2 percent is sufficient) solution for 1 to 10 days before planting. Soaking of seeds should be done in a very shallow container so that the seeds are not deprived of oxygen. Seed treatment methods should be used only for seeds that germinate quickly. In the case of slow-germinating seeds, the treatment should be deferred until the seeds commence active germination, in which case the treatment is fundamentally the same as with seedlings.

2. Seedling treatment. Freshly germinated seeds are kept immersed in about 0.2 percent colchicine solution in a shallow container, or placed on filter paper or blotting paper spread out in shallow dishes and thoroughly wet with the solution, for 3 to 24 hours or longer, depending upon how rapidly the seedlings are growing and how bulky they are. If the material is suitable, the young seedlings may be treated as follows: The root ends placed on a strip of absorbent cotton that is thoroughly wet with water and then the seedlings rolled into a bundle. The cotton covers the root ends and forms a plug that will fit loosely in a small vial. The bundle of plants is then inverted and set in the vial with only the stem ends immersed in the water solution of colchicine. By this method the root system is kept moist only with water and practically unaffected by the chemical, thus preventing some of the mortality that usually follows treatment of whole seedlings. When seeds are germinated in the dark the resulting seedlings, being longer than normal, are often suitable for this type of treatment.

Another method is: Plant seeds, well spaced, in pots or flats. When seedlings have emerged from the soil and the cotyledons or the first one or two leaves have opened, place a small amount of 0.2 to 0.5 percent colchicine solution between the cotyledons or over the vegetative growing point. To each 10 cc. of colchicine solution in water or 10 percent glycerine-water solution should be added 1 to 2 drops of 10 percent solution of Santomerse-SX (a product of the Monsanto Chemical Company, St. Louis, Missouri) or a similar wetting-detergent agent, such as

Dreft, a household detergent, at the same concentration, used as a substitute for soap.<sup>2/</sup> Leave some seedlings untreated for comparison with the treated ones. The growth of the treated seedlings will be retarded in comparison with the untreated ones if the treatment is at all effective.

3. Treatment of growing shoots and buds. Tips of rapidly growing shoots, or an entire growing bud, may be treated by placing a drop or more of the colchicine solution over the tip, once or several times, or by immersing the growing tip for a number of hours in a vessel containing the solution. The tips of growing shoots may be smeared with a mixture of 0.5 to 1.0 percent colchicine in lanolin. All these methods have given satisfactory results, particularly with herbaceous plants. Woody and semiwoody plants (buds or young shoot tips) require a somewhat different treatment. For these, one of the following solutions is recommended: (1) 0.5 to 1 percent colchicine in a 10 percent water solution of glycerine; or (2) 0.5 to 1 percent water solution of colchicine. In order to facilitate penetration and spreading of these solutions, a very small amount of a wetting or spreading agent (see above) should be added.

Another method is: Cut off the very tip of a vigorously growing shoot and wet with the colchicine solution the upper three lateral buds, three to eight times at 1 or 2 day intervals. Other buds either should be dug out or shoots growing from untreated buds should be destroyed. Colchicine effect should be looked for in those buds or shoot tips that are partly retarded in growth as a result of the treatment. The effectiveness of the treatment may be judged from the distorted growth of first few leaves emerging after the treatment. If no such distortion is observed in the lower leaves of the growing shoot, it is most obvious that colchicine solution has not penetrated into the treated buds at all.

It is advisable to limit the treatment to buds of one young shoot on a cutting or grafted shoot; the rest should be cut off. Shoots that are to be treated should be allowed to grow some 6 inches or more before the tip or lateral buds are treated. The older leaves on such shoots should be left to furnish nutrition for the plant.

In woody material, if not in herbaceous plants as well, total polyploidy affecting the whole new growth very seldom occurs. The effect often may be confined to a narrow sector on a branch, or to a certain tissue, or to a limited portion of tissues. When shoot growth from treated buds does not show any retardation as compared with growth from nontreated material, it may indicate that the treatment has not been effective. Basilar leaves of a shoot grown from a treated bud appear distorted in varying degrees, when the treatment has been effective.

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<sup>2/</sup> Mention of companies or products is not to be construed as an endorsement of these firms or products by the U. S. Department of Agriculture.

Colchicine-induced polyploidy can be often recognized in leaves that are denser in texture, darker in color, larger and broader than normal, or in part of a leaf (often a half, one side of the midrib) that is larger than the other part and denser in texture. Such leaves should be looked for in the upper portion of the shoots that develop from the main growing point of the treated tips or from axillary buds that were present in the treated regions. Polyploid branches may be grown from buds in the axils of the changed leaves by cutting off the part above these leaves. If no change is detected, then buds that were very young and were developing at the time of treatment and which may subsequently have remained dormant may be forced into growth on a possible chance that they have been affected by the treatment and contain polyploid changes. Any extraneous growths that may hinder growth in the treated part should be constantly eliminated.

When a polyploid sector is discovered in an otherwise normal stem, it should, if possible, be forced to grow out into a branch; the propagation of such a branch by grafting or rooting (depending on the type of plant) should not be delayed. A polyploidized sector as such may not continue to grow indefinitely parallel with a normal tissue in the same stem, and eventually may be lost as the stem develops.

4. Treatment of bulbs and corms. The terminal growing points in bulbs and corms are buried within a mass of scales or other storage tissues and are, therefore, difficult to reach with colchicine. In the case of tulips, narcissus, bulbous iris, and onion the colchicine may be introduced into the growing regions by a hypodermic needle. In gladiolus, penetration of colchicine solution into small cormels has been achieved under a partial vacuum. This method might prove effective also with small bulbs of tulip and narcissus. The "eyes" on the gladiolus bulbs may be treated directly, in the same way buds are treated. In bulbaceous material such as lilies, where detached scales from the bulb may readily form small bulbs at the base after planting, the following treatment has been very successful: Scales are detached and immediately immersed in a 0.2 percent solution for about 2 hours; then they are planted with the tips exposed. The length of time of treatment and concentration of the solution may have to be varied to obtain results with the different species of lilies and other bulbaceous material.

5. Treatment of day-lilies and similar types of plants. Many herbaceous plants form large root stocks or crown from which new buds arise each year. These new buds originate from cells within but near the surface of the crown tissue, and if colchicine is present at the time bud differentiation is initiated, some of the buds may be expected to give rise to polyploid shoots. The daylily is such a plant and may be treated by cutting the tissue back deep into the crown and scooping out a shallow basin in which the colchicine solution can be placed. It has been found that colchicine in concentrations of 0.1 to 0.5 percent have

been effective. The colchicine has been used in water solutions and in lanolin. It should also be effective in 10 percent glycerine. Colchicine-lanolin paste is applied only once. It is advisable to repeat the treatment with colchicine solution in water or glycerine every few days for three to four treatments to be sure enough of the drug enters the crown of the plant. As the new buds develop, some will be affected as evidenced by their slow growth as compared to unaffected buds. The latter should be removed as soon as possible to force the polyploid bud into growth.

If polyploid shoots are obtained, it must be remembered that they are on the old diploid root system. Such shoots may form small side shoots at the nodes and these should be removed and rooted in order to obtain a plant with a polyploid root also.

At this point it must be emphasized that desirable changes in many plants are not to be expected, since some of them are already polyploids and further doubling of the chromosomes may result in inferior plants. Also, it cannot be emphasized too strongly that colchicine treatment frequently has been unsuccessful, especially with woody plants and other material where penetration of the chemical into the growing regions presents difficulties; and furthermore, that the doubled-chromosome forms, if produced, are not always improvements over the normal types. Some changes often can be recognized only by careful observation, involving the use of a microscope or a high-magnifying hand lens. Some changes effected by colchicine may be so inconspicuous that they at times escape casual observation. In interpreting results from the use of colchicine it is not only necessary to know the growth habits and cultural requirements of the experimental plant but also to have expert knowledge of plant structure, some knowledge of the principles of genetics, experience in recognizing and evaluating induced changes, and training in the use of the microscope. A high-magnifying hand lens in some cases may make it possible to see a change in size and distribution of stomates in comparison with stomates of a normal leaf. Changes in superficial structures such as hairs and glands that are of epidermal origin may also be detected. No trace of colchicine is expected to be present in parts of polyploid plants resulting from the experiments.

As can be seen from the above presentation, colchicine cannot be considered as a plant food, "growth elixir," or fertilizer. Its application to induce polyploidy is confined mostly to vegetative growing points of individual plants, and only a very limited number of plants is treated at a time. For a more extended discussion of artificially induced polyploidy and information on some of the results obtained, readers are referred to the limited list of literature references at the end of this paper, particularly to reference 4, "Colchicine Polyploidy and Technique", which is essentially a general review of the literature on the subject. The publications listed may be found in the libraries of most State agricultural colleges and experiment stations.

NOTE: Colchicine may be purchased from following chemical concerns:<sup>3/</sup>  
Inland Alkaloid Company, Tipton, Indiana; Mallinckrodt Chemical Works,  
New York City; Eimer & Amend, New York City; Merck & Co., Inc., Rahway,  
New Jersey; S. B. Penick & Company, 132 Nassau Street, New York City;  
Gane and Ingram, Inc., 43 W. 16th Street, New York City; Bios Laboratories,  
607 West 43rd Street, New York 18, New York

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3/ On furnishing the names of manufacturers of colchicine, it is impracticable to provide a complete list. These references are given for the convenience of the reader, with the understanding that no discrimination is intended and no guarantee implied by the U. S. Department of Agriculture. In order to find out who has colchicine available for sale in small quantities, one should write to all of the above companies, because some may have discontinued handling it.

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